EFFECTS OF HYPOBARIC HYPEROXIA ON THE DEVELOPMENT AND ACTIVITY OF IMMUNE SPLEEN CELLS

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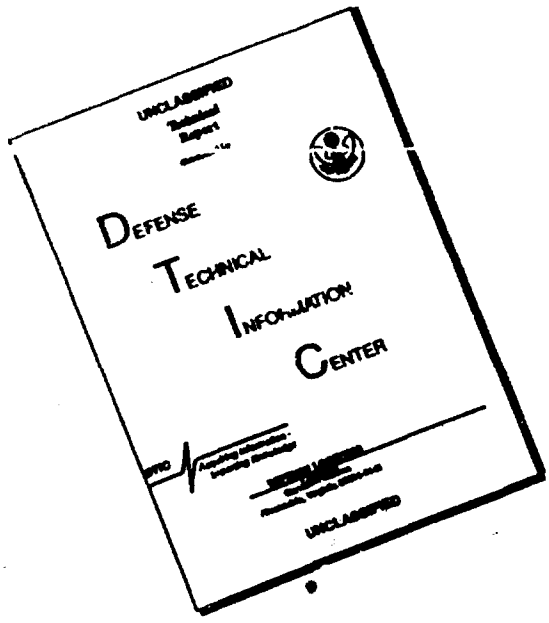

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13. ABSTRACT

The purpose of this study was to evaluate the effect of intermittent exposure to hypobaric hyperoxic conditions (380 mm Hg and 100% O_2) on cellular aspects of resistance to an infectious disease. Mice were immunized against Franciscella tularensis while being exposed to the test environment. Spleen cells from these immunized-exposed donors were transferred to nonexposed recipients that were maintained in a normal ground-level environment. These recipient mice were then challenged with F. tularensis to compare their resistance with that of control animals receiving spleen cells from immunized donors which had not been exposed to the test environment. The reverse was also accomplished by immunizing groups of donor mice -aintained at ground level and challenging the recipients (of their spleen cells) that were exposed to the test environment. The results indicate that exposure of the donor animals to hypobari. hyperoxi a did not affect the development of cellular immunity; but, in exposed recipients, the activity of immune cells was impaired.

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Effects of Hypobaric Hyperoxia on the Development and Activity of Immune Spleen Cells

TONY D. DAVID and JEROME P. SCHMIDT

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The purpose of this study was to evaluate the effect of intermittent exposure to hypobaric hyperoxic conditions (380 mm Hg and 100% O_2) on cellular aspects of resistance to an infectious disease. Mice were immunized against Franciscella tularensis while being exposed to the test environment. Spleen cells from these immunized-exposed donors were transferred to nonexposed recipients which were maintained in a normal ground-level environment. These recipient mice were then challenged with F. tularensis to compare their resistance with that of control animals receiving spleen cells from immunized donors which had not been exposed to the test environment. The reverse was also accomplished by immunizing groups of donor mice maintained at ground level and challenging the recipients (of their spleen cells) which were exposed to the test environment. The results indicate that exposure of the donor animals to hypobaric hyperoxia did not affect the development of cellular immunity; but, in exposed recipients, the activity of immune cells was impaired.

PUBLISHED REPORTS concerning the effects of exposure to modified environments on host resistance to infectious disease are difficult to interpret and evaluate because of the variations in test conditions and the numerous infectious agents employed by different investigators. Extrapolation of results has been further hindered by the complexity of host-parasite relationships and the lack of knowledge concerning specific cellular defense mechanisms. Although the exact mechanisms involved are not well-defined, it is quite evident that modified barometric pressures and gaseous atmospheres do alter host resistance to various pathogenic microorganisms.[2-6,8-14] The present study was designed to evaluate environmental effects on cellular immunity. Tularemia in the mouse was selected as the model because resistance to that disease in this host is primarily dependent upon phagocytic cells.[1]

MATERIALS AND METHODS

Environmental Test Conditions: The hypobaric hyperoxic test environment (380 mm Hg and 100% O_2 was maintained in an altitude chamber having an interconnecting airlock and work space. The temperature was constantly controlled at 20°C, and the relative humidity ranged from 40% to 60%. The mice were otherwise maintained in an adjacent well-ventilated animal room with a constant temperature of 20°C.

Experimental Groups: In each of our two studies, animals were separated into four groups, designated respectively as A-A, A-G, G-A, and G-G. The "A" signified exposure to the test environment (hereafter referred to as "altitude") 4 hrs per day for 14 consecutive days; and "G" represented ambient ground conditions. In one study (Table I), donor mice were separated into the four groups for immunization against tularemia. Group A-A animals were subjected to altitude for 14 days and then given their first immunization. After 14 more days of altitude conditioning, they were given their second immunizing dose. Group A-G animals were acclimatized similarly, but were retained at ground level after the first 14-day period. Group G-A was a reversal of group A-G, and group G-G was maintained at ground level throughout the immunization period. To allow time for maximal response to the second injection, exposure to altitude (groups A-A and G-A) or ground level (groups A-G and G-G) was continued for 10 more days before the animals were sacrificed and their spleens harvested. Spleen cells from these four groups of immunized donors were transferred to recipient animals which were maintained at normal ground conditions. The four groups of recipient animals were identified according to the environmental conditioning of their donor animals, and were challenged 24 hrs after the cell transfer. Each group was composed of 20 mice and these were separated into two cages of 10 mice each.

The same procedure was used in the other study (Table II) except that the recipients, instead of the

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TABLE I. THE EFFECTS OF HYPOBARIC HYPEROXIA ON DEVELOPMENT OF IMMUNE CELLS.*

	Exposure schedule of donors**			Challenge of ground-level recipients**			
Groups	Before first immunization (14 days)	Before second immunization (14 days)	Before cell harvest (10 days)	Mortality Dead/total	%	Mean survival time (Days)	Survival Index
A-A	altitude	altitude	altitude	20/20	100	5.32	38.0
A-G	altitude	ground level	ground level	14/20	70	5.96	59.8
G-A	ground level	altitude	altitude	11/20	55	5.50	66.6
G-G	ground level	ground level	ground level	12/20	60	4.92	61.1
Pooled standard deviation					31.1	1.87	22.2

*Differences ($p < 0.1$) in resistance between the environmental test groups (A-A, A-G, and G-A) and the control group (G-G) were *not* detected.

**Spleen cells from the altitude-exposed donors were transferred to the recipients that were maintained at ground level. The transfer was made 24 hrs prior to challenge of the recipients.

donors, were exposed to the test environment. Donors (which are not represented in Table II) were immunized as in the previous study, but maintained under normal ambient conditions. Potential recipient animals were separated into four groups for environmental conditioning. After 14 days of exposure to altitude, groups A-A and A-G received spleen cells and, 24 hrs later, were challenged with *Franciscella tularensis*. Animals not previously subjected to the chamber environment were treated similarily and then separated into groups G-A and G-G. After the spleen cell transfer and challenge, groups A-A and G-A continued to be exposed to altitude, and groups A-G and G-G were maintained at ground level.

Bacteria, Immunization, and Cell Transfer: The Live Vaccine Strain (LVS) and the highly virulent (SCHU S4) strain of *F. tularensis* were grown on cystine glucose blood agar.[7] Bacteria from 48- to 72-hr cultures were suspended in physiologic saline with 0.1% gelatin for animal inoculation. Turbidity and plate counts were used to attain the desired concentration of microorganisms in 0.1 ml amounts for injection. The challenge-dose received by recipients in the first study (Table I) was 10 colony forming units (CFU) of the SCHU S4 strain. The infectious challenge for recipients in the second study (Table II) was increased to 100 CFU of the SCHU S4 strain.

Donor mice, 8 wks of age or older, were immunized by infection with the LVS strain. Immunization consisted of an intraperitoneal injection of 125 CFU and, 14 days later, a booster dose of 10,000 CFU. In preliminary work less than 10 CFU of the LVS strain proved to be as lethal as 1,000 CFU. Although shorter survival time was associated with the higher dosage, the end mortality rate was not dose-dependent. To prevent mortality among donor animals, they were treated with 400 μg of streptomycin subcutaneously for 3 consecutive days, beginning 2 days after each immunizing dose.

Donor animals were anesthetized with methoxyflurane and killed by exsanguination. Siliconized glassware was used throughout the preparation of the cells for transfer. The spleens were aseptically removed and placed in petri dishes containing cold Hanks' Balanced Salt Solution (BSS), pH 7.2-7.4, with 100 μg of dihydrostreptomycin and 100 units of penicillin/ml. Spleens from eight to 10 mice were pooled for processing to yield approximately 10^8 cells/ml of the final dilution to transfer to recipient animals.[1] Suspensions were prepared by cutting each spleen in five or six pieces and then expressing the splenic pulp in a loose-fitting tissue homogenizer with 5 ml of BSS. This preparation was filtered through a 250-mesh stainless-steel sieve into a beaker containing an additional 5 ml of BSS. The cells were then sediment-

TABLE II. THE EFFECTS OF HYPOBARIC HYPEROXIA ON THE EXPRESSION OF CELLULAR IMMUNITY.

	Exposure schedule of recipients*					
Groups	Before challenge (14 days)	After challenge (14 days)	Mortality Dead/total	Percent	Mean survival time (days)	Survival Index
A-A	altitude	altitude	30/30	100†	4.00§	28.6††
A-G	altitude	ground level	30/30	100†	4.13	29.5††
G-A	ground level	altitude	30/30	100†	4.25	30.4**
G-G	ground level	ground level	19/20	95	4.47	35.3
Pooled standard deviation				2.7	0.48	1.5

*Spleen cells from donors maintained at ground level were transferred to recipients 24 hrs prior to challenge.

†Differs from group G-G $p < 0.1$

§Differs from group G-G $p < 0.05$

**Differs from group G-G $p < 0.01$

††Differs from group G-G $p < 0.005$

ed at 500 g for 5 to 10 mins in a graduated 15-ml conical test tube. They were washed again and resuspended in BSS without antibiotics for intraperitoneal injection. Treatment of the cells with streptomycin was necessary to insure against infection of the recipient mice with residual bacteria of the LVS strain. Total cell counts were made to determine if the desired number of spleen cells per ml had been obtained. The percentage of viable cells was determined with 0.5% trypan blue in physiologic saline; cells that excluded the dye were considered viable.

Animals: Inbred C57BL male mice were used to insure the compatibility of spleen cells that were transferred from donor to recipient animals. The mice were further characterized by serologic testing to exclude latent infections of pneumonia virus of mice, reovirus 3, Theiler's mouse encephalomyelitis (GD-VII), K virus, polyoma virus, Sendai virus, murine adenovirus, and mouse hepatitis virus (MHV). An immune response to the LVS strain by cell donors was confirmed by the presence of antibodies against *F. tularensis*. The microorganism was recovered from 10% of the animals that died. Criteria used for identification were cultural characteristics, morphology and staining properties. Histologic examination was performed on animals from each test group. The histopathologic changes observed were, consistently, a necrotizing lesion of lymphatic tissues and viscera.

Statistical Evaluation of Data: In both studies, the mortality rate, survival time, and survival index of groups A-A, A-G, and G-A were compared with those of the appropriate G-G control group. During each study the animals were observed for 14 days after infection, and deaths were recorded at 12-hr intervals. The values were compared using analysis of variance techniques. Probability levels of < 0.1 are presented, but only differences with probability levels of < 0.05 were considered significant.

RESULTS AND DISCUSSION

The first study (Table I) involved challenging the recipients maintained at ground level. Thus, the A-A, A-G, G-A, and G-G group designations correspond to the exposure schedule of the donor animals. The results revealed no alterations in the development of cellular immunity attributable to the environmental exposure of the donors. However, exposure of the recipient animals to altitude in the second study (Table II) did have a pronounced effect; and, in terms of survival time, group A-A was less resistant than the G-G control group. The survival index of each of the three groups (A-A, A-G, and G-A) was significantly smaller than that of the G-G control group.

The basic difference in the design of the two studies was that: In the first study, donor animals were subjected to the test environment; and, in the second study, the recipients were subjected to the test environment. Results from these two experiments were expected to indicate whether the development or activity of immune phagocytes had been most affected. The resistance of mice receiving immune spleen cells from exposed donors was not different from that of recipients of cells from nonexposed donors. Therefore, the development of cellular resistance was probably not affected. On the other hand, exposure to the chamber environment did reduce the survival index of animals receiving immune spleen cells derived from nonexposed donors. Hence it can be inferred that the *in vivo* protective activity of immune cells was impaired in the exposed mice, or that the hypobaric hyperoxia caused the recipients to be less capable of deriving passive protection from transferred spleen cells. The exact mechanisms involved were not defined, but cellular resistance appears to have been affected by the environment of the host.

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